Remarks

Claims 11, 17-18, 22 and 24 are here amended, and claims 1-10, 12, 19-21, 26-27, 50 and 52 have been canceled. Claims 11, 13-18, and 22-25 are currently pending in the application. Applicants reserve the right to pursue canceled and withdrawn claims in this or future applications.

Claim 11 has been amended to have the limitations of canceled claim 12. Claim 11 is further amended to more distinctly claim the subject matter regarded by Applicants as their invention. Support for the amendment can be found in the original description and claims, as described in detail below. No new matter has been added.

Claims 17 has been amended to depend on a currently pending claim. Claim 22 has been amended for antecedent basis.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**".

Applicants believe that the claims herein are in condition for allowance, which is respectfully requested.

The subject matter of claim 11 as amended is well supported. As a preliminary matter the application provides examples within the scope of the claims. In particular, the description is replete with instances of assaying expression in a population of muting nucleic acid-treated cells, immediately after the population has been provided with a muting nucleic acid and in the absence of any selection for the transfection of a genetic marker. See for example, p.5, showing steps of transforming animal cells, and then assaying the transformed cells for changes in expression; see also p. 7, lines 26-28 (assay of endogenous mRNA in untransfected or transiently transfected cells). The limitation, "the nucleic acid being double stranded or non-complementary with respect to mRNA associated with the endogenous gene" is well supported, as shown below.

The description with numerous working examples consistently shows immediate use of muting nucleic acid-treated cells, absent any selection prior to further use. This is important, because the method of the present invention pointedly omits any requirement for at least one and preferably two selecting drugs. See p. 8, lines 13-16, and lines 26-29: and p. 9 lines 11-12 and 19-21. See also definitions of "transfection" and "electroporation" on p. 11, lines 17-23, and discussion of the transient condition of a vector on p. 11, lines 10-12.

See the transfection procedure referenced on p.16. lines 3-18, and p. 6, line 6, incorporating by reference a standard methods paper on this topic.

Given these examples, it is abundantly clear to a person of ordinary skill in the art of molecular genetics and genetic manipulation of cells, that inhibition of gene expression in the cells by addition of muting nucleic acid is achieved without any selection for the presence of muting nucleic acid. (Indeed, a person of ordinary skill in the art would know that selection in animal cells is not even possible with the muting nucleic acid disclosed in the application.)

For this reason, the disclosure of the application in this regard satisfies 35 U.S.C.§112. See for example, *W.L. Gore Assoc. v. Garlock, Inc.*, 220 U.S.P.Q.303, 315, 721 F.2d. 1540, 1556 (Fed.Cir.1983), which states that "[p]atents are written to enable those skilled in the art to practice the invention, not the public." See also, *Atmel Corp. v. Information Storage Devices Inc.*, 53 U.S.P.Q.2d 1225, 1230, 198 F.3d. 1382, which states, "[t]he specification would be of enormous length if one had to literally reinvent and describe the wheel."

It is unnecessary for a patent to point out what is omitted in a procedure. In *S3 Inc. v. Nvidia Corp.*, 59 U.S.P.Q.2d 1745, 2001 WL 876905 at p.5, the court (in a decision dated Aug. 3, 2001) pointed out: "...patent documents need not include subject matter that is known in the field of the invention...patents are written for persons experienced in the field of the invention."

Support for limiting independent claim 11 to double stranded nucleic acid is replete throughout the application. See the description, for example, at p. 15 lines 2-3, and all of p. 17, describing standard recombinant manipulations performed using well-known double stranded nucleic acid. The application is replete with description of transfection of cells using plasmids of well characterized and documented origin, which are known by one of ordinary skill in the art of recombinant technology to be double stranded. See for example p. 22, lines 26-27.

Claim 11 alternatively requires that the nucleic acid be non-complementary with respect to mRNA associated with the endogenous gene. Support for this leg of the claim is found in the discussion of the working examples. These examples include testing transfected cells according to the methods of the invention for *de novo* synthesis of anti-sense nucleic acid, thereby showing that this is not the mechanism of gene muting. See the description, p.

15, lines 1-3, and p. 29, lines 8-9 for support for muting nucleic acid that is not complementary to mRNA.

The claims as amended are free of the prior art

Applicants have amended independent claim 11 so that this method is now directed a method for muting expression of an endogenous gene in a cultured population of animal cells. The method comprises the steps of: (a) identifying a muting nucleic acid composition having a sequence that is homologous to a sequence in the endogenous gene, the nucleic acid being double stranded or non-complementary with respect to mRNA associated with the endogenous gene; and (b) delivering the muting nucleic acid into the population of cells under conditions devoid of a selection for integration of the nucleic acid into a chromosomal site, so that expression of the endogenous gene in the population as a whole is inhibited even though such gene's sequence is not therein disrupted.

Applicants acknowledge the Examiners' comment, in Paper 20 dated October 9, 2001, that the newly proposed claim limitations would eliminate enablement concerns over knockout technology, and thank the Examiners. Further discussion herein of knockout technology is thereby deemed unnecessary.

The claims as amended are not directed to antisense technology

In addition, claim 11 as amended is free of prior art antisense technology. Element (a) of amended claim 11 is "identifying a muting nucleic acid composition having a sequence that is homologous to a sequence in the endogenous gene, the nucleic acid being double stranded or non-complementary with respect to mRNA associated with the endogenous gene". As claim 11 is so amended, other pending claims that are dependent on claim 11 are so amended.

Antisense technology by definition uses nucleic acid that is complementary to mRNA transcribed from the target gene. Since claim 11 requires that the muting nucleic acid be double stranded or non-complementary with respect to mRNA associated with the endogenous gene, the muting nucleic acid of claim 11 as amended is free of antisense technology.

Applicants respectfully request that the Examiner withdraw rejections of the claim under 35 U.S.C. 102 and 35 U.S.C. 103 with respect to anti-sense technology.

The written description satisfies 35 U.S.C. 112 first paragraph

The Advisory Action of Oct. 9, 2001, alleges that the specification does not provide working examples that demonstrate muting in vivo. However, the working examples do in fact provide a range of muting of gene expression. For example, Example 5 on p. 21 of the application, lines 22-23 states, "...the level of endogenous procollagen mRNA was surprisingly greatly reduced." Further, p. 22 in the same example, lines 2-3 state, "...transcripts of the endogenous gene, although greatly reduced in amount, were clearly visible." Further, p. 22, lines 28-29 state, "...the level of endogenous collagen mRNA was about 7% that of the control cells." Additional examples of various quantities resulting from muting of endogenous gene expression can be found throughout the specification.

Applicants assert that muting of an endogenous gene in a population of cells, referring to reduction or elimination of gene expression, devoid of selection for a rare event such as gene insertion and gene disruption, has been more than adequately described and exemplified.

The Examiners allege that only one working example is provided to muting a single nucleotide sequence. Applicants point out that additional prophetic examples, showing that additional sequences could be muted, are provided in the application as filed (see Example 15, pp. 31-33, providing enablement for muting of the HIV *tat* gene, muting of the gene for TNFα, and muting of immunoglobin encoding genes associated with autoimmune diseases. Applicants assert that one of ordinary skill in the art of molecular biology could, with the instructions provided and without undue experimentation, perform such muting operations.

Applicants further have discovered at least ten recent publications citing the invention as published by the inventors, an example of which was previously made of record in this application. The ability of many others to reproduce the present invention in other systems proves that the method of muting as described herein has proved to be predictable.

Applicants urge the Examiner to withdraw rejection of the claims under 35 U.S.C. 112 first paragraph.

The written description satisfies 35 U.S.C. 112 second paragraph

Claims as amended conform to the requirements of 35 U.S.C. 112 second paragraph.

Use of the term "substantially transient" in claim 26 has been obviated by cancellation of this claim.

The Examiners state that the term "population" is indefinite because it is not clear which cell types are encompassed by the term. Applicants assert that the term population, used to indicate a plurality if not all or the vast majority of cells in a culture, is not vague, for the reasons that follow.

The expression, "population of cells" is a term of art known to all of ordinary skill in the art of microbiology and cell biology. In claim 11, use of the term "population" is particularly suited to describe the response of a large number of cells to the muting nucleic acid, by inhibiting gene expression throughout the culture, rather than by selecting a rare cell that has engaged in an insertion event on a chromosome. Applicants assert that this expression properly describes the element of "delivering" the muting nucleic acid so that expression of the endogenous gene in the population of cells is inhibited.

The description as filed includes a variety of different mammalian cell lines, some of which are transformed cells (see p. 13, line 28-p. 14, line 2). Applicants assert that a variety of different clonally pure recipient cell types, including transformed cells, revertants of the transformed cells, and non-transformed or normal cells, are provided. It is well known by those of ordinary skill in the art of cell biology that Rat-1 cells are normal fibroblasts, and that v-*fos* cells are oncogenically transformed cells.

Further, the description as filed indicates that the population of cells is transfected at very high multiplicity (hundreds to thousands of copies of plasmids per cell; see p. 21, line 28; see p. 23, line 25 and Figure 3B). Use of a term from the Poisson distribution enables the cell biologist to calculate the proportion of cells of a population that by chance are not transfected. The multiplicity determined from the working examples in the present description, together with the Poisson calculation, prove that the vast majority of cells in the working examples are transfected with numerous plasmids, and that untransfected cells are so rare as to be nonexistent.

For these reasons, the term population as used in the present claims is understood by one of ordinary skill in the art of cell biology to mean a set of cells that have been similarly treated, and therefore this term is distinct and clear.

Applicants assert that claim 11 and the claims dependent thereon meet the requirements of 35 U.S.C. 112 second paragraph, and respectfully request that the rejections of the claim on this basis be withdrawn.

Summary

In view of the foregoing amendments and remarks, Applicants submit that the claims are now in condition for allowance. Early and favorable reconsideration of the application is therefore respectfully solicited. The Examiners and the Practice Specialist are invited and encouraged to contact Applicants' representative at the telephone number below if such contact would assist in expediting the present application to allowance, and prior to issuance of any action other than allowability.

It is believed that a fee for a three month extension of time has already been paid, as has a fee for a Notice of Appeal. Applications thereby request that any other fee required for the timely consideration of this amendment be charged to Deposit Account No. 19-4972.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

11. (Twice amended) A method for muting expression of an endogenous gene in a cultured population of animal cells, the method comprising the steps of:

(a) [providing] identifying a muting nucleic acid composition having a sequence that is homologous to a sequence in the endogenous gene, the nucleic acid being double stranded or non-complementary with respect to mRNA associated with the endogenous gene; and

(b) delivering the muting nucleic acid into the population of cells under conditions devoid of a selection for integration of the nucleic acid into a chromosomal site, so that expression of the endogenous gene in the population as a whole is inhibited even though such gene's sequence is not therein disrupted.

17. (Twice amended) A method according to claim [12] 11, wherein the muting transgene sequence is [substantially] homologous to an endogenous sequence [that extends to] comprising a portion of the endogenous gene selected from at least one of the group of: [the] a 5' untranscribed portion, [the] a transcribed coding portion including introns, [the] a 3' untranslated portion, [the] a 3' untranscribed portion, and a portion that overlaps adjacent ends of at least two portion of the endogenous gene.

18. (Amended) A method according to claim 17, wherein the nucleic acid comprises a sequence [that is substantially] homologous to an endogenous sequence located in the 5' portion of the endogenous gene.

22. (Twice amended) A method according to claim [11] 17, wherein the muting nucleic acid comprises a sequence that is [substantially] homologous to an endogenous sequence located at the 3' portion of the gene.

24. (Twice amended) A method according to claim 11, wherein [the step of] delivering the muting nucleic acid in (b) is selected from the group of: transforming, transfecting, electroporating, infecting, and lipofecting the nucleic acid into the cells [at a

plasmid copy number which is a multiple of the number of cells to which the nucleic acid is delivered].